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Original Article

Seroepidemiological Study of Human Hydatidosis in Meshkinshahr District, Ardabil Province, Iran

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ABSTRACT

Background: The aim of this study was to conduct a sero-epidemiological survey in Meshkinshahr, Ardabil Province, northwestern Iran to detect the rate of hydatidosis in the city and nearby villages. Literature shows that no such study has been conducted so far.

Methods: Overall, 670 serum samples were collected from 194 males and 476 females from patients referred to different health centers of the region. All patients filled out a questionnaire and an informed consent. Sera were analyzed using indirect-ELISA test. Ten µg /ml antigens (Antigen B derived from hydatid cyst fluid), serum dilutions of 1:500 and conjugate anti-human coombs with 1:10000 dilutions were utilized to perform the test. Data analysis was conducted using SPSS software ver. 11.5.

Results: The seroprevalence of human hydatidosis was 1.79% by ELISA test in the region. This rate for females was 1.68% and males 2.6%, respectively. There was no significant difference as regards all factors studied and the seropositivity. According to job, farmers and ranchmen had the highest rate of infection as 3.17%. The sero-prevalence of infection was 2.6%% in illiterate people which showed the highest rate. As regards residency, urban life showed no significant difference with rural life (1.1% vs. 2.58%). Age group of 69-90 yr old, with 4.62% as prevalence had the highest rate of positivity.

Conclusion: Obtained sero-prevalence of hydatidosis shows more or less a resemblance to other cities of Iran, although due to the specific condition of the city we expected more rate of sero-positivity.

Keywords: Seroepidemiology, Human hydatidosis, ELISA, Iran

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Introduction

Echinococcosis or hydatidosis, caused by the larval stage of *Echinococcus granulosus*, is considered as the most fetal and important helminthic diseases throughout the world (1). The burden of the disease is very high and causes dramatic damages in terms of human and veterinary affairs (2).

Iran is regarded as an endemic region of hydatidosis and the disease has been reported more or less throughout the country (3, 4). Knowing the prevalence of the disease in each country is of high importance, so that the necessary measures for eradication or control of the disease could be conducted. So far, five countries have been able to eradicate hydatidosis (2). The previous studies have shown the prevalence of human hydatidosis as 0.6-1.2/100000 population in Iran (3). Dogs play a critical role in transition the hydatidosis. The rate of infection with *E. granulosus* in stray dogs in Iran shows a prevalence of 5% to 49% in different parts of Iran (3), so it is expected that we have the disease in areas where dog owners are found in considerable amount. Meshkinshahr is a city with agricultural and anima husbandry condition and dogs are found due to the necessity of the situation. Survey of literature shows that so far no study has been conducted to clarify the situation of human hydatidosis in this area. Moreover, previous studies have demonstrated that from year 1946 to 1993, 37 cases of human alveolar echinococcosis were reported from Iran, which nearly all of them belonged to this region (3). In addition to this helminthic disease, Meshkinshahr is regarded as one the most important cities in Iran, as regards the visceral leishmaniasis or kala-azar, a fetal protozoan disease.

The aim of the present study was to detect the seroprevalence of human hydatidosis in Meshkinshahr, Ardabil Province, northwestern Iran using ELISA test and to determine the risk factors involved in spreading the disease.

Materials and Methods

Serum samples were collected from 670 cases including 188 males and 483 females referred to clinical and health centers, as well as private and governmental laboratories in Meshkinshahr, Ardabil Province, northwestern Iran. The city is of 3617 km^2, with 164007 populations at the last census in 1996. The distribution of the population in terms of urban and rural residency is equal. Sera were stored in refrigerator at -20 °C, and then were sent to Dept. of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran for examination with ELISA. Although based on the statistician consulting the volume of sample was determined as 458, but for more caution, overall 670 people were enrolled in the study. To select the cases, from each geographical region of the city and nearby villages, sampling was conducted using different clusters and simple sampling. In each cluster form the villages of High, Koojengh, Youssef Kahn, and Naghdi Olia and four regions of the city including Gharehdarvish, Sayyedabbad, Farhangian and Parasil sampling was conducted according to the proposal.

An informed consent was taken from all subjects, besides; a questioner was filled out for each case including various factors such as age, sex, job, locality, literacy etc.

Antigen

Hydatid cyst fluid (HCF Ag) was aspirated from hydatid cysts obtained from livers and lungs of sheep slaughtered at the local abattoir. Antigen B was purified and extracted as

stated earlier (5), and was utilized as follows.

ELISA test

ELISA test was performed in 96 well microplates (Nunc, Denmark) as previously described (5), with some modifications. Microplate wells were coated overnight at 4 °C with 100 µl HCF Ag (10 µg/ml) in 0.05 M bicarbonate buffer, pH 9.6. Wells were washed 3 times in PBS plus 0.05% Tween 20 (PBS-T) and blocked with PBS-T containing 1% BSA for 30 min at 37 °C. Sera were added at 1:500 dilutions in PBS-T, incubated at 37 °C for 1 h then washed as before. Antihuman IgG-HRP (Sigma Chemical Co., Poole, Dorset, United Kingdom) conjugates were added at 1: 10000 dilutions in PBS-T and the microplate incubated and washed as before. This was then developed in OPD substrate (5 mg 1, 2-phenylenediamine, 12.5 ml of 0.2 M citrate phosphate buffer pH 5, 10 µl 30% H2O2). The absorbance was read at 492 nm after 10 min using an automatic microplate reader (State Fax® 2100, Awareness, USA). Altogether 30 samples of sera from healthy volunteers had been collected during the previous studies were examined to set the cut-off. Cut- off was calculated as mean + 3 SD.

Data analysis

All data were analyzed using SPSS software ver. 11.5. P value less than 0.05 was considered as significant.

Results

The seroprevalence of human hydatidosis was detected as 1.79% by ELISA test in the region. Cut-off was detected as 0.32 (Fig. 1). The prevalence of hydatidosis among females was 1.68% and males 2.6%, which showed no significant difference.

The rate of the disease was 3.6% in people having history of contacting with dog but 0.71 in people with negative history in this regard. No significant difference was seen. As regards job, farmers and ranchmen had the highest rate of infection as 3.17%, followed by 2.8% in people with free jobs and 1.76% in housekeepers. No significant difference was seen on this topic. The prevalence of infection was 2.6% in illiterate people, which showed the highest rate with no significant difference.

The highest rate of infection was found in 60-90 yr old age group with 4.62% and the least rate was in 10-19 yr old age group as 0% (Table 1). There was not significant difference between age group and positivity.

In terms of residency, the rate of infection in urban and rural areas was 1.1% and 2.58%, respectively, but no significant differences were detected. Singles and married cases included 0.74% and 2.05% of the positivity with no significant difference. The prevalence of disease in people with and without history of geophagia was 3.57% and 1.71%, respectively, but no significant difference was seen. Having positive or negative history of unwashed eating raw vegetable encompassed 3.88% and 1.41% seropositivity in examined sample with no significant difference.

The prevalence of disease in people with history of having any especial diseases other than hydatidosis and without it was 7.14% and 1.67%, in that order which showed no significant difference. This rate in people with history of having surgery was 2.59%, while in people not having it was 1.55% with negative significant difference.

Table 1: Distribution of positive cases of hydatidosis using ELISA according to age group (yr) in Meshkinshahr, Ardabil Province, Iran

Age group (yr)	Sample taken (No.)	Positivity (No.)	Sero-Prevalence (%)
00-09	61	1	1.63
10-19	56	0	0.00
20-29	96	1	1.04
30-39	141	1	0.70
40-49	117	2	1.70
50-59	91	2	2.19
60-69	108	5	4.62
Total	670	12	1.79

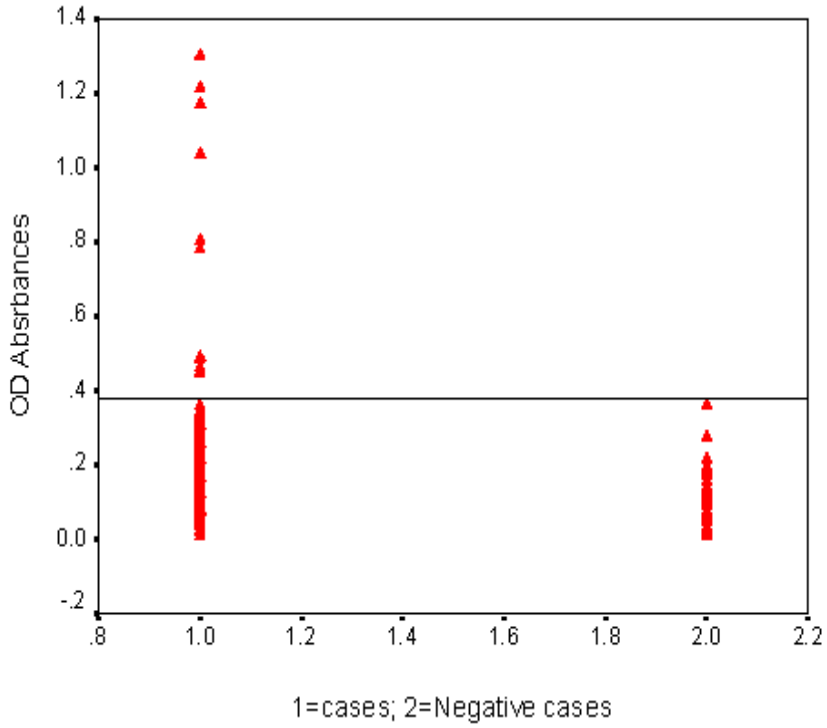

Fig. 1: Analysis of sera from subjects and normal controls by IgG-ELISA. Serum samples obtained from patients with hydatidosis (Lanes 1), normal controls (Lanes 2)

Discussion

Iran is regarded as an endemic area for hydatidosis and many studies, whether hospital or seroepidemiological based, have demonstrated the disease throughout the country to different extents (3, 6-10). Hydatidosis is responsible for approximately 1% of admission to surgical wards (11). A comprehensive hospital based study in Iran shows the over-

all prevalence as 2083 cases form 2001-2005 (12). Using seroepidemiology a rate of 1.2%-21.4% of infectivity is obvious in different parts of the country (3). The annual incidence rate of hydatidosis in human in Hamadan as 1.33 /100,000, in Kashan 3/100,000 populations, in Babol 1.18/100,000, and throughout Iran 0.61/100,000 are among documented reports [References in (3)].

Regarding this fact that Iran encompasses a considerable amount of patients infected with hydatidosis and something must be done to control the disease at the first stage, it is necessary to be aware of the rate of infection in different parts of the country. Our team is supposed to determine the prevalence of human hydatidosis in Iran using seroepidemiology method and among cities, which we have, data now is Meshknishahr, Ardabil Province. As mentioned earlier in this text, this city if of high importance in terms of some considerable diseases such as visceral leishmaniasis, alveolar echinococcosis etc, and many studies have been conducted so far in the area (13, 14). However, considering the lack of information on human hydatidosis, we selected it to determine the rate of infection using ELISA.

Overall, a prevalence of 1.79% as for human hydatidosis was detected in Meshknishahr. In comparison to previous similar studies conducted in Iran, it was lower than stated in Zanjan as 3% (15), Kurdistan 7.3% (16), Kashan 2.04% (8), Golestan 2.34% (6), Khuzestan in nomads, 13.8% (17), Tehran 5.9% (7), and Chaharmahal va Bakhtyari 4.8% (18) but higher than reported in Ilam as 1.2% (19). These data shows the reality that the rate of infection is lower than most parts of Iran, regardless that other important diseases are found in the area. It is possible that the way of life and observing the health regularities are among factors determining this.

Regardless other studies, which showed housekeepers as the highest number of infected with hydatidosis (6, 8, 19), our study showed the farmers and ranchmen (4.76%), encompassed the highest rate. This might be due to the culture of the area, where men are in more contact with risk factors than women are. The rate of infection was higher in male than females in our study (2.06% vs. 1.68%).

In our study, age group of 60-90 had the highest rate on infection as 4.62%. In Arbabi et al. study, the highest rate was in 60-80 yr age group (8), in Sedaghat Gohar et al. in 20-30 yr (7), and in Baharsefat et al. study in 30-60 yr (6). However, different studies show different results, which depends on the situation of the region, kind of calculating data, culture etc.

Regarding other factors such as contact with dog, geophagia, literacy, urban or rural area, our study shows more or less similar coincidence with other studies conducted in Iran (6-8). Factors, which showed significant difference among different groups, were including history of contact with dog, level of education, and marital status.

In conclusion, this study might be able to complete another part of puzzle as for human hydatidosis in Iran. Completion this puzzle is necessary in terms of preparing the first steps to conduct a decisive program for controlling or eradication of the disease. As some countries have been able to eradicate human hydatidosis from their countries, so Iran potentially may be able to do this, provided that all circles of the chain are provided scientifically and in due time.

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